Plasma homocysteine as a risk factor for atherothrombotic events in systemic lupus erythematosus

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Summary

Background The aim of this study was to asses whether plasma homocysteine is a risk factor for stroke and other thrombotic events in patients with systemic lupus erythematosus (SLE)—a condition known to be associated with premature atherothrombotic complications.

Methods In this prospective study, we investigated the association between homocysteine and risk of stroke and thrombotic events in 337 SLE patients in the Hopkins Lupus Cohort Study, with follow-up of 1619 person-years (mean 4·8 [SD 1·7] years). Each patient had four follow-up assessments per year to obtain information about established risk factors for thrombosis and coronary artery disease. The prospectively defined endpoints were occurrence of stroke and arterial or venous thrombotic events between 1987 and 1995. Blood samples were taken at study entry from fasting patients. Plasma homocysteine, folate, vitamin B12, and pyridoxal 5'-phosphate (PLP) concentrations were measured. Raised homocysteine concentrations were defined as more than 14·1 μmol/L.

Findings 93% of the study population were women, 54% African American, and 45% white. The mean age of participants was 34·9 (SD 11·7) years. During follow-up there were 29 cases of stroke and 31 arterial thrombotic events. Raised homocysteine concentrations were found in 51 (15%) SLE patients. The log-transformed total homocysteine concentrations correlated with serum folate (r=0·31, p=0·0001). In univariate analyses, raised homocysteine concentrations were significantly associated with stroke (odds ratio 2·24 [95% CI 1·22–4·13], p=0·01) and arterial thrombotic events (3·74 [1·96–7·13], p=0·0001). After adjustment for established risk factors, total plasma homocysteine concentrations remained an independent risk factor for stroke (2·44 [1·04–5·75], p=0·04) and arterial thromboses (3·49 [0·97–12·54], p=0·05).

Interpretation Homocysteine is a potentially modifiable, independent risk factor for stroke and thrombotic events in patients with SLE.

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Introduction

Cerebrovascular, coronary, and peripheral vascular thromboembolic events are major causes of morbidity and mortality in systemic lupus erythematosus (SLE). Risk factors for thrombotic events in SLE include hypercoagulation caused by antiphospholipid antibodies (lupus anticoagulant or anticardiolipin), active lupus with vasculitis, and other vascular disease risk factors such as hypertension and hyperlipidaemia; male patients are more susceptible than female patients. The main cause of coronary artery disease in SLE is premature atherosclerosis,[1] though myocardial infarction and coronary ischaemia due to thrombus without atherosclerosis,[2] vasculopathy,[3] and vasculitis[4] have also been reported. In a previous prospective case-control study, we found that duration of prednisone use, age, hypertension, morbid obesity, and hyperlipidaemia were predictors of coronary artery disease in SLE.[5]

Homocysteine is a non-essential, sulphur-containing aminoacid produced as a result of transamination of aminoacids during normal metabolism.[6] An association between homocysteine and premature atherosclerosis has been found in familial homocysteinaemia.[7] The biological mechanisms involved may include a direct toxic effect of homocysteine on endothelium[8] and indirect effects, such as induction of a vascular-endothelial-cell activator,[9] promotion of vascular smooth-muscle proliferation, and an inhibitory effect on endothelial cell growth.[10] Several studies have found an association between mild hyperhomocysteinaemia and occlusive vascular disease.[7,11] In addition, the Physicians' Health Study[12] and the Framingham Heart Study[13] have shown that high homocysteine concentrations are associated with increased relative risk of coronary artery disease, stroke, and carotid vascular disease. Perry and colleagues'[14] case-control study of homocysteine concentration and risk of stroke in middle-aged men found that homocysteine concentrations were significantly higher in men with stroke than in controls selected from the same background; adjustment for other risk factors did not attenuate this association.

We carried out a prospective study of the association between plasma homocysteine concentration and atherothrombotic events in patients with SLE in the Hopkins Lupus Cohort Study—a prospective study of the risk factors for morbidity associated with SLE and its treatment. Since women with SLE are at risk of premature thrombotic and atherosclerotic events, there was a high rate of thrombotic events in our study sample.

Methods

The Hopkins Lupus Cohort Study, founded in 1987, includes all patients referred to the rheumatology clinics at the hospital who have a clinical diagnosis of SLE and give informed consent to participate in the study. Each patient has four standard clinical and laboratory assessments per year; all patients were assessed by

	Number of participants		p
	With stroke (n=29)	Without stroke (n=308)	
Demographic characteristics			
Female/male	25 (86%)/4 (14%)	286 (92·9%)/22 (7·1%)	0·26
White/African American	14 (48%)/15 (52%)	138 (45%)/165 (55%)	0·85
Mean (SD) age in years	38·6 (15·2)	34·5 (11·3)	0·17
Mean follow-up (years)	5·27 (1·55)	4·75 (1·70)	0·11
Risk factors			
Total cholesterol >5·1 mmol/L	22 (76%)	187 (60·7%)	0·39
Body-mass index >27·8 kg/m²	7 (22%)	97 (31·5%)	0·50
Hypertension	17 (59%)	122 (39·6%)	0·05
Diabetes	3 (10%)	21 (6·8%)	0·45
Serum creatinine >88·4 μmol/L	13 (45%)	116 (37·7%)	0·17
Currently taking prednisone	22 (76%)	177 (57·5%)	0·11
Clinical characteristics (mean [SD])			
Russell viper venom time (s)	31·10 (1·3)	28·80 (1·2)	0·09
Anticardiolipin antibody (log titre)	1·16 (1·73)	1·10 (2·08)	0·66
Total homocysteine (μmol/L)	10·26 (1·91)	7·41 (1·88)	0·009
Folate (nmol/L)	13·80 (4·33)	15·68 (4·10)	0·27
Vitamin B12 (pmol/L)	310·10 (1·51)	310·39 (1·15)	0·99
Pyridoxal 5'-phosphate (pmol/mL)	12·20 (4·2)	16·90 (3·0)	0·24

Number of participants vary because of missing data. *Fisher's exact test or *t* test after log transformation.

Table 1: **Baseline distribution of characteristics of SLE patients according to whether or not stroke occurred**

one rheumatologist (MP). At the time of this study, 337 SLE patients were in the cohort with 1619 person-years (mean 4·8 [SD 1·7] years) of follow-up. At each follow-up assessment, information about other risk factors for thrombosis and coronary artery disease was obtained (eg, weight, hypertension, and serum creatinine, glucose, and antiphospholipid antibody concentrations).

The prospectively defined primary endpoints were occurrence of stroke and arterial or venous thrombotic events between 1987 and 1995. Venous thrombotic events were defined as deep-venous thrombosis (confirmed by venogram) or Budd-Chiari syndrome. Arterial thrombotic events were defined as: thromboembolic (but not haemorrhagic or vasculitic) stroke (confirmed by computed tomography or magnetic resonance imaging); myocardial infarction (confirmed by electrocardiography and a rise in the MB fraction of creatine kinase); gangrene of the fingers (clinical diagnosis, and not due to vasculitis on arteriogram); or other arterial thrombosis. Obesity was defined as a body-mass index greater than 27·8 kg/m² for men and 27·3 kg/m² for women.[15] Diabetes was defined by American Diabetes Association criteria.[16] Three patients each had stroke and deep-venous thrombosis at different times during the study and were included in both endpoint groups. No patient had more than one stroke or arterial thrombotic event during the observation period.

Blood samples were taken at study entry from fasting participants between 0800 h and 1000 h. The blood samples were centrifuged at 2500 *g* for 15 min, and plasma was separated, and stored at –80°C until analysis. One portion of each sample was used to measure the total homocysteine concentration in plasma and the concentrations of the vitamins that affect homocysteine metabolism (folate, vitamin B12, and pyridoxal 5'-phosphate [vitamin B6, PLP]). Total homocysteine concentration in plasma was measured according to the method of Araki and Sako.[17] Raised homocysteine concentrations were defined as a fasting plasma homocysteine concentration of more than 14·1 mmol/L.[18] Vitamin B12 concentrations were measured by a radioassay (Ciba-Corning, Medfield, MA, USA). PLP concentrations were measured by a modification of the tyrosine decarboxylase method of Camp et al.[19] Plasma folate concentrations were measured by a microbial assay with a 96-well plate and manganese supplementation as described by Tamura et al[20] and a radioassay kit (Ciba-Corning). The interassay coefficients of variation were

9% for homocysteine, 7% for vitamin B12, 16% for PLP, and 13% for folate.

We measured the lupus anticoagulant in platelet-poor plasma by the modified Russell viper venom time or recalcified clotting time.[21] Anticardiolipin was measured by a previously validated polyclonal assay.[22]

The distribution of each continuous variable was examined graphically and statistically for normality. Total homocysteine, PLP, folate, vitamin B12, and anticardiolipin antibody concentrations, and Russell viper venom time were positively skewed and a natural logarithmic transformation was applied before analysis. Categorical data were analysed by Fisher's exact test; continuous variables were analysed by *t* test after logarithmic transformation. Scatterplots and Pearson correlation coefficients were used to analyse the association of total homocysteine, vitamin B12, and serum creatinine concentrations with age. Point biserial correlations were used to measure the association between total homocysteine concentrations and the primary endpoints of stroke, venous thrombosis, and arterial thrombosis. For each outcome, univariate logistic regression was used to calculate odds ratios with 95% CI for homocysteine and other risk factors. Multivariate logistic regression was used to analyse the association beteen total homocysteine concentration and the primary endpoints, after adjustment for established risk factors for thrombosis—age, sex, race, obesity, hypercholesterolaemia, hypertension, diabetes, renal insufficiency, and presence of the lupus anticoagulant (Russell viper venom time was more strongly associated with thrombotic events in univariate analysis than anticardiolipin antibody, and thus should provide a more conservative estimate of the effect of total homocysteine concentration). The significance of the β coefficients was tested by the likelihood ratio.

Population attributable risk percentage (%PAR)—a measure of the proportion of excess thrombotic events in the study population attributable to raised total homocysteine concentrations—was calculated according to the formula:

$$\%PAR = \frac{P\,(RR-1)}{P\,(RR-1)+1} \times 100$$

where RR is the relative risk estimated from the odds ratio, and P is the proportion of the population likely to benefit from a reduction in total homocysteine concentrations.[23]

The protocol was approved by the Human Investigations Committee of Johns Hopkins University.

Results

Clinical, demographic, and biochemical characteristics of participants who had or did not have a stroke are shown in table 1. 324 (96·1%) of the cohort met four or more of the American Rheumatism Association (American College of Rheumatology) classification criteria for SLE.[24] There were no differences in age between the sexes or races. 199 (59·1%) of the SLE patients were currently taking prednisone (median dose 6 mg [IQR 0–15]). 71 (21·0%) of patients were taking hydroxychloroquine (standard dose 400 mg daily).

Total homocysteine concentrations above the cut-off of 14·1 μmol/L were found in 51 (15%) SLE patients. The distribution of total homocysteine concentrations was positively skewed (figure). Log transformation of homocysteine gave a normal distribution. Total homocysteine concentrations were higher in men than in women (10·5 [SD 1·9] *vs* 7·4 [1·7] μmol/L, p=0·005); in patients with serum creatinine concentrations of 88·4 mmol/L or more than in those with lower values (10·0 [1·8] *vs* 6·3 [1·8] μmol/L, p<0·0001); in patients taking prednisone than in non-users (8·2 [1·8] *vs* 6·7 [1·9] μmol/L, p<0·008); and in those who had an abnormal Russell viper venom time than in those with normal values (9·31 [1·9] *vs* 7·12 [1·8] μmol/L, p<0·0001). No
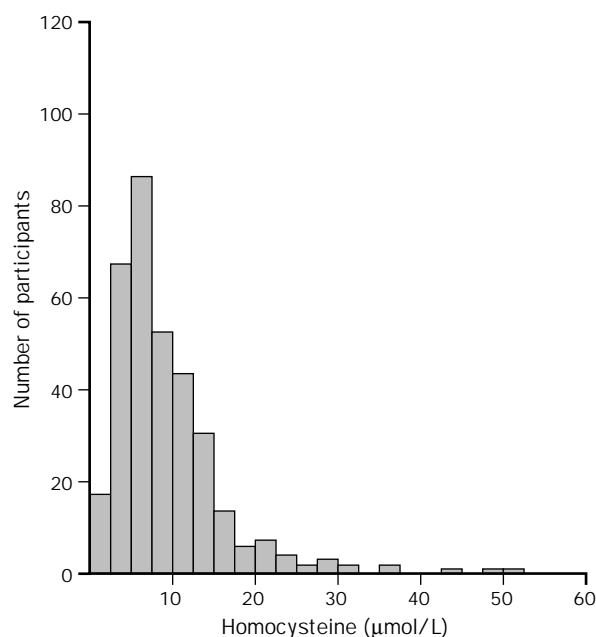
Figure: **Distribution of total homocysteine concentrations in study population**

association was found between total homocysteine concentration and race or use of hydroxychloroquine. Low concentrations of plasma folate ($r=-0.39$, p=0.0001) and PLP ($r=-0.20$, p=0.0001) were associated with high concentrations of plasma homocysteine. There was no association between vitamin B12 concentrations and raised concentrations of homocysteine ($r=0.06$, p=0.254).

There were 29 (8.6%) strokes, 31 (9.2%) arterial thrombotic events, and 33 (9.8%) venous thrombotic events during the 8 years of follow-up. In univariate analyses, the log-transformed concentration of homocysteine was significantly associated with risk of stroke (odds ratio=2.24) and arterial thrombotic events (3.74), but not with venous thrombotic events (1.15; table 2). By contrast, no such association was found between thrombotic events and blood concentrations of folate, PLP, or vitamin B12.

Homocysteine remained a significant predictor of stroke and of arterial thrombotic events in a multiple logistic regression model, which included all the variables used in univariate analysis (table 3). A change of one unit in log-transformed plasma homocysteine concentrations (equivalent to a 2.7-fold increase) increased the odds ratio for stroke and thrombotic events to 2.4 and 3.5, respectively. The % PAR associated with homocysteine was 17.3% for stroke and 27.3% for arterial thrombosis. Plasma homocysteine concentration was not significantly

associated with anticardiolipin antibodies, Russell viper venom time, or lupus anticoagulant. Homocysteine was not an independent predictor of venous thrombotic events in multivariate analysis.

Discussion

Previous studies have shown that raised circulating homocysteine concentrations are a risk factor for several thrombotic and atherosclerotic disorders in the elderly—for example, carotid artery stenosis,[13] peripheral vascular disease,[25] stroke,[26] and myocardial infarction.[12] These findings are important because high blood concentrations of homocysteine are both common and modifiable. The Framingham Heart Study found that 29.3% of the study population had homocysteine concentrations of more than 14.1 μmol/L, and low plasma concentrations of folate, vitamin B12, and PLP accounted for 67% of the cases of high homocysteine.[18] Hyperhomocysteinaemia increases with age, and because the Framingham population is now elderly (median age 75 years) the implications of the association between plasma concentrations of vitamins and homocysteine and vascular disease for younger individuals are not clear. To examine further this hypothesis, we used a younger study population known to be at increased risk of accelerated atherosclerosis and thrombosis.

In this cohort, we found that plasma homocysteine was associated with plasma folate and PLP, but not with vitamin B12. All three vitamins are known to affect the metabolism of homocysteine, and were associated with homocysteine concentrations in the Framingham population.[18] However, low concentrations of vitamin B12 may occur in the elderly because of poor absorption of the vitamin due to atrophic gastritis; but this disorder is rare among younger people such as those in our SLE cohort.[27] Although we found an association between plasma homocysteine concentrations and folate and PLP, on the one hand, and between plasma homocysteine concentrations and vascular thromboses, on the other, there was no association between concentrations of folate, vitamin B12, and PLP and thrombotic events. This lack of association suggests that a complex interaction underlies the vitamins' effect on plasma homocysteine concentrations. In addition, our study may not have had sufficient power to detect an association between the vitamins and thrombosis. Nonetheless, our findings of an association between plasma homocysteine and folate and PLP accords with the hypothesis that hyperhomocysteinaemia in SLE may be treatable with a combination of these vitamins. Our findings may also have implications for other populations at risk of accelerated

Risk factors	Stroke (n=29)		Venous thrombosis (n=34)		Arterial thrombosis (n=31)	
	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
Age per year	1.03 (1.00–1.06)	0.08	1.00 (0.97–1.04)	0.99	1.06 (1.03–1.09)	0.0001
Male sex	2.07 (0.65–6.66)	0.2	3.03 (1.12–8.16)	0.03	3.82 (1.37–10.27)	0.01
Race (African American=1, white=0)	1.12 (0.51–2.42)	0.8	1.23 (0.60–2.53)	0.60	1.48 (0.66–3.34)	0.4
Log homocysteine concentration	2.24 (1.22–4.13)	0.01	1.15 (0.66–2.00)	0.7	3.74 (1.96–7.13)	0.0001
Cholesterol >5.1 mmol/L	1.67 (0.65–4.26)	0.3	2.13 (0.78–5.78)	0.14	2.68 (0.90–8.01)	0.08
Body-mass index >27.8 kg/m²	1.44 (0.56–3.75)	0.5	1.63 (0.65–4.10)	0.3	1.41 (0.53–3.77)	0.5
Hypertension	2.16 (1.00–4.68)	0.05	2.91 (1.39–6.11)	0.005	6.35 (2.51–16.04)	0.0001
Diabetes	1.58 (0.44–5.64)	0.5	1.30 (0.37–4.61)	0.7	2.30 (0.73–7.27)	0.15
Serum creatinine >88.4 μmol/L	1.74 (0.80–3.79)	0.16	1.22 (0.59–2.52)	0.6	4.66 (1.91–11.37)	0.0007
Anticardiolipin antibody	1.39 (0.62–3.07)	0.43	1.05 (0.50–2.17)	0.9	1.15 (0.99–4.66)	0.05
Russell viper venom time per s	1.03 (0.99–1.07)	0.10	1.04 (1.00–1.08)	0.02	1.02 (0.98–1.06)	0.04

Odds ratios are for one unit change in the independent variable.

Table 2: **Univariate analysis of risk factors for thrombosis events**

Risk factors	Stroke			Arterial thrombosis		
	β-coeficient	Odds ratio (95% CI)	p	β-coeficient	Odds ratio (95% CI)	p
Age per year	0·0090	1·01 (0·97–1·05)	0·67	0·0530	1·05 (1·01–1·10)	0·02
Sex (male=0, female=1)	−0·4020	0·67 (0·15–2·98)	0·61	−0·6760	0·51 (0·08–3·07)	0·48
Race (African American=1, white=0)	−0·0099	0·99 (0·37–1·67)	0·98	−0·6294	0·54 (0·14–1·96)	0·34
Body-mass index >27·8 kg/m²	−0·5205	0·59 (0·21–1·67)	0·31	0·2911	1·34 (0·37–4·87)	0·65
Cholesterol >5·1 mmol/L	0·5906	1·81 (0·55–5·90)	0·31	1·7550	5·78 (0·65–51·52)	0·06
Hypertension	0·0228	1·04 (0·33–2·89)	0·97	1·4171	4·13 (0·76–22·27)	0·07
Diabetes	0·9742	2·65 (0·54–12·93)	0·25	1·3147	3·73 (0·30–47·05)	0·26
Serum creatinine >88·4 µmol/L	0·0290	1·03 (0·35–3·05)	0·96	0·8444	2·33 (0·52–10·41)	0·25
Log homocysteine concentration	0·8932	2·44 (1·04–5·75)	0·04	1·2505	3·49 (0·97–12·54)	0·05
Russell viper venom time per s	0·0264	1·02 (0·97–1·09)	0·38	−0·1067	0·99 (0·90–1·09)	0·86

Odds ratios are for one unit change in the independent variable.

Table 3: **Multivariate analysis of risk factors for stroke and arterial thrombosis**

atherothrombotic events, such as patients with renal failure, but data about these populations are not currently available.

We found that 15% of the cohort had high blood concentrations of homocysteine; men and participants with raised serum creatinine concentrations had higher homocysteine concentrations than other SLE patients. Although the association between serum creatinine and homocysteine may reflect reduced renal clearance of homocysteine in patients with renal lupus, the association between prednisone and homocysteine is not clear. Perhaps prednisone increases homocysteine concentrations directly through its hormonal effect. Alternatively, use of prednisone is a marker for severe disease and may reflect reduced clearance of homocysteine due to slightly abnormal renal function, not manifested by raised serum creatinine.[28]

After adjustment for established risk factors plasma homocysteine remained an independent predictor of stroke and arterial thrombosis in patients with SLE. The size of this effect was substantial: an increase of one log unit of homocysteine concentrations led to a 2·4-fold increase in the risk of stroke, and a 3·5-fold increase in the risk of arterial thrombosis. This finding accords with Boushey and colleagues' meta-analysis, which reported that raised homocysteine concentrations[23] caused an odds ratio of 2·5 for stroke. The large size of the effect of homocysteine in this study may reflect the lack of effect of other, more established, risk factors in our study population, due to the young age of participants and the high proportion of women. The % PAR associated with homocysteine in our cohort was 17·3% for stroke and 27·3% for arterial thrombosis, which is similar to that of 18·4% for stroke calculated from the data of Boushey et al[22]—for this calculation we used the conservative assumption that only the 15% of our study population with raised homocysteine concentrations would be likely to benefit from vitamin therapy.

Our finding of an association between homocysteine and risk of stroke accords with the results of Perry et al,[14] but differs from those of Verhoef and colleagues' study,[26] which found no such association. The lack of an association between homocysteine and venous thrombosis suggests that there is some specificity for the arterial circulation in the association between homocysteine and thrombosis in SLE patients. den Heijer and colleagues[18] found, by contrast, an association between venous thrombosis and homocysteine in patients with recurrent deep-venous thrombosis.

Our data provide the first evidence of a potentially modifiable risk factor for stroke in young patients with SLE. Because of the increased rate of atherothrombotic events in this population, our study was able to assess the association between homocysteine concentrations and risk of vascular disease in a younger population than has previously been reported. Furthermore, the effects of thrombosis in young patients are potentially more devastating than in the elderly, thus, the possibility that vitamins could prevent thrombotic events in SLE deserves attention. Studies of vitamin intervention for thrombotic events in patients with SLE are currently under way.

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Cost implications of random mandatory drugs tests in prisons

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Summary

Background Compulsory urine testing of prisoners for drugs, a control initiative, was introduced in eight prisons in England and Wales early in 1995. Despite no evidence of effectiveness, testing was extended to all prisons in England and Wales by March 1996. We consider the cost of testing.

Methods We combined the costs of refusals, confirmatory tests, punishment of confirmed positives for cannabis or for class A drugs to estimate the average costs of random compulsory drugs testing. These costs were then compared to: i) the healthcare budget for a prison; and ii) the cost of putting in place a credible prisons' drugs reduction programme. We then used Scottish data on incarceration and regional prevalence of injecting drug users to estimate the extent of the injecting drug use problem that prisons face.

Findings Costs per 28 days of the random mandatory drugs testing control initiative in an establishment for 500 inmates where refusal rate is a) 10% or b) nil; and 35% of urine samples test positive, one tenth of them for class A drugs were estimated at between a) £UK22 800 and b) £UK16 000 per 28 days [a) $US35 100 and b) $US24 600]. This cost was equivalent to twice the cost of running a credible drugs reduction and rehabilitation programme, and around half the total healthcare expenditure for a prison of 500 which averaged £UK41 114 per 28 days [$US64 860]. Major cost-generating events were the punishment of refusals—over one third of cost a)—and

testing positive for cannabis—over 50% of cost a). In Scotland, around 5% of injecting drug users (IDUs) are incarcerated at any time: 5% of Lothian's drugs care, treatment and prevention costs and 2·5% of its HIV/AIDS prevention budget in 1993–94 amounted to £UK101 300 per annum—or £UK7770 per 28 days ($US11 970)—and about 35% of monthly MDT costs.

Interpretation We suggest that 5% of current resources for drugs prevention and treatment and for IDU-targetted HIV/AIDS prevention should be directed towards the prisons because in the prisons, where 5% of the clients are at any time, injectors have less access to harm reduction measures than on the outside.

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Introduction

Compulsory urine testing for drugs in prisoners was introduced in eight prisons in England and Wales—including Holloway (a women's prison)—early in 1995. Neither ethical and scientific arguments against compulsory tests as 'a means of gathering information'[1] nor operational objections[2] dissuaded the Prison Service from extending its 'war on drugs' to all prison establishments in England and Wales by March 1996.

The 1994 Criminal Justice and Public Order Act permits both random and 'on suspicion' selection of prisoners from whom a urine sample can be required and tested for drugs. During the pilot phase of mandatory testing, random sampling has been organised from Prison Service headquarters. Refusal to provide the required urine sample breaches prison rules and is punishable by 28 days' loss of remission. Prisoners whose urine sample tests positive for cannabis only are liable to punishment of 14 days' loss of remission, and 21 days if their sample is positive for class A drugs such as heroin.

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